The present invention relates to a microscale binding assay, an analyte-binding array, and a kit for use in a mass-sensing binding assay with a high sensitivity for very low quantities of analyte. All three independent claims, 1, 23, and 26, require: microscopic size of the sorbent zones, substantial depletion of analyte from the sample, and concentration of the depleted analyte on the microscopic sorbent zones. These three limitations are crucial for the successful implementation of the mass-sensing assay of the present invention. As explained on page 7, lines 21-31, because the present assay concentrates substantially all analyte present in a defined sample volume, the same amount of analyte is harvested, for example, by the array from 100 µl of 10^{-13} M analyte solution and from 10 µl of 10^{-12} M analyte solution. Accordingly, the optical signal generated by analyte bound to the sobent zone is the same in both cases. Therefore, by sensing mass instead of concentration, the instant assay greatly increases sensitivity of the analyte detection. Since the instant assay of the present invention harvests analyte from samples of a well-defined volume, the concentration of the sample can be easily calculated based on the volume of the sample applied and the analyte mass harvested.

Ekins, on the other hand, teaches the direct measurement of the sample concentration, which is independent of sample volume. Because Ekins method is concentration-based, it is less sensitive at lower concentrations than the present method.

The Examiner acknowledged that the '202 patent does not teach the analyte being substantially depleted from the sample, but relied on the Immunoassay reference for teaching the same. Applicants respectfully disagree with the Examiner's reading of the Immunoassay reference.

As it has been explained in detail in the response to the previous Office Action, the Immunoassay reference does not teach substantial depletion of analyte present in the defined sample volume as required by claims 1, 23, and 26. The term "substantial depletion" has a well-defined meaning in the present specification. In accordance with the teachings of the present invention, the depletion is substantial when "at least about 60% of the analyte will be captured by a high affinity binding partner" (page 14, lines 17-22) and when a bulk analyte concentration in the sample is maximally perturbed (page 7, lines 21-31).

The Immunoassay reference, on the contrary, teaches only an insignificant and localized analyte depletion in the medium immediately surrounding the analyte-binding sites. In fact, the Immunoassay reference explicitly requires that a proportion of analyte bound by antibody is so

small that "the resulting reduction in ambient analyte concentration can be ignored" (page 173, left column). Furthermore, the Immunoassay reference teaches that the maximum number of antibody-binding sites in the microspot causes "insignificant disturbance (<1%) to the analyte concentration in the sample" (page 173, right column). Therefore, the Immunoassay reference teaches away from depleting substantially all analyte from the sample.

The Examiner alleges that the Immunoassay reference teaches substantial depletion because it states on page 173, paragraph 1: "Analyte binding by antibody clearly causes analyte depletion in the surrounding medium." However, the cited phrase represents only a portion of a sentence and does not reflect its true contextual meaning. The complete sentence that includes the cited phrase by the Examiner reads: "Analyte binding by antibody clearly causes analyte depletion in the surrounding medium, but provided the proportion bound is small, the resulting reduction in ambient analyte concentration can be ignored." Moreover, in the same paragraph, the Immunoassay reference additionally states: "For example, if the amount of sensor-antibody used is less than 0.01/K, then analyte depletion in the medium is invariably less than 1%, and the system is effectively sample volume independent." Therefore, the analyte binding fails to deplete 99% of the available analyte from the sample. Accordingly, the Immunoassay reference does not teach substantial analyte depletion as stated by the Examiner.

The Examiner also alleges that that the Immunoassay reference teaches substantial depletion because Figure 4 of the reference shows "antigen bound concentrations as high as 100% when using higher antibody concentration." Applicants disagree.

The Immunoassay reference states that Figure 4 illustrates ambient analyte assay (page 172, right column). In particular, Figure 4 illustrates that in ambient analyte assay a "fractional occupancy of antibody binding sites [by an analyte] solely reflects the ambient analyte concentration and is independent both of sample volume and of the amount of antibody in the system" and page 173, column). Those skilled in the art would recognize that a fractional occupancy is independent of a sample volume and amount of antibody only when a limited number of analyte molecules bind to antibodies and, thus, the bulk concentration of the sample is not perturbed. In fact, the Immunoassay reference itself states that a system is independent from a sample volume when the "analyte depletion in the medium is invariably less than 1%" (page 173, left column). Moreover, the Immunoassay reference explicitly limits the maximum total number of antibody molecules to a such number that causes "insignificant disturbance (<1%) to

the analyte concentration in the sample." Based on these teachings, those skilled in the art would be at least discouraged from binding 100% of analyte as the Examiner suggests. Therefore, Figure 4, considered in view of the explanatory comments made in the Immunoassay reference, does not teach or suggest a substantial depletion of the analyte from the sample.

The Examiner also argues that applicants did not provide scientific data showing that unexpected results were achieved. Applicants respectfully disagree.

As explained in the previous response, the present invention provides an unexpected benefit of high signal-to-background ratio of binding assay by concentrating the signal on a small area of the support. This benefit is derived from the confinement of the binding partner to a small area of support to maximize analyte-binding capacity per unit area.

For example, as explained on p. 8, lines 2-23, and as demonstrated in Figure 1 of the present invention, the mass assay of the present invention has about 10-100 times higher analyte-binding capacity than the ambient assay of the Immunoassay reference. In Figure 1, the horizontal axis shows the molar concentration of the analyte sample applied to a microscopic zone and the vertical axis shows the number of molecules bound by a single microscopic zone per 100 μl of the sample. For each analyte concentration, an amount of the analyte bound from a 100 μl sample is calculated based on Ambient Analyte model of Ekins and Mass Assay model of the present invention. The resulting graphs show 10-100 times higher binding capacity of the arrays of the present invention as compared to those of Ekins, and, thus, 10-100 times higher sensitivity of the assay of the present invention as compared to the assay of Ekins.

These calculations and results are further confirmed by comparing experimental data of the present invention with experimental data provided in Ekins' references. In this regard applicants would like to draw the Examiner's attention to Example 1 of the present invention and Example 1 of the '202 patent. Example 1 of the present invention describes Biotin/avidin binding system. In that system about 10^{10} dye-biotin molecules are bound per printed spot with a diameter of 200μm (page 20 lines 7 and 32-33). The area of such spots can be easily calculated ($0.785 \times D^2$) and equals about 0.02 mm^2 ($160\mu m^2 = 160 \times 10^{-6}$ mm^2). Therefore, in the present invention, the binding capacity of the printed spots is about 10^{12} per mm^2 of area.

Example 1 of the '202 patent teaches that spots having an area of $1mm^2$ contain 5×10^9 molecules of antibody and, thus, have an analyte-binding capacity of 5×10^9 per mm^2 of the spot area. Therefore, the analyte-binding capacity of the spots of the '202 patent is almost three

orders of magnitude less than in the present invention. Because the spots of the '202 patent have much lower analyte-binding capacity and, thus, cannot concentrate analyte, a weaker fluorescent signal is generated. In fact, Figure 1 of the present invention demonstrates that the signal of Ekins ambient assays is from 60 to 100 times weaker than the signal produced in assay of the present invention.

Applicants also argued in the previous response that the instant invention is not obvious in view of the cited art because the assay of the present invention senses mass, whereas the Ekins assay measures analyte concentration. Applicants explained that, in the present invention, the analyte depletion is so substantial that it maximally perturbs the bulk concentration of analyte in the sample. As a result, the developed signal reflects the total analyte mass contained in the defined sample volume and harvested onto the microscopic measurement spot (page 7, lines 21-31). The Immunoassay reference, on the contrary, teaches only an insignificant and localized analyte depletion in the medium immediately surrounding the analyte-binding sites. Such minuscule analyte depletion does not appreciably affect the overall bulk concentration of the analyte in the sample solution (page 173, left column). Accordingly, the developed signal is indicative of ambient analyte concentration, but not the total analyte mass in the sample.

In response to this argument, the Examiner alleges that the present invention measures concentration rather than the total bound mass. The Examiner appears to believe that the instant invention uses "the same method steps, the same laser microscopy techniques to assay the analyte, and provides results in terms of molecules bound." Applicants disagree.

As explained on pages 20, lines 11-21, line 6, the arrays of the present invention respond to analyte mass, not to concentration, because they have sufficient affinity and binding capacity to deplete the analyte contained in a sample. Figure 6 demonstrates such mass-sensing nature of the present invention. Figure 6 shows experimental results from three-hour incubations in which 100 microliter aliquots of $5x10^{10}$M DBCY5-biotin solution were in contact with arrays having 1, 9, 25, 49, or 70 spots. When the number of avidin spots per array is varied, it is expected in a mass-sensing array that the density of analyte bound per spot would vary inversely with the number of spots present. This is exactly the result shown in Figure 6: when fewer spots are printed per array, the total analyte mass present during incubation is collected onto fewer spots, leading to an increased signal from any one spot. Therefore, contrary to the Examiner's belief, the present invention does provide a mass-sensing assay unlike the cited references.

In light of the foregoing, applicants respectfully submit that the '202 patent and the Immunoassay reference, either alone or in combination with other known techniques of the art, cannot make claims 1, 23, and 26 obvious. None of the cited references, either alone or in combination, would have motivated one skilled in the art to arrive at the present invention, which requires a substantial depletion of the analyte from the bulk solution and concentration of the analyte on the microscopic sorbent zones. Claims 2-4, 13-19, 24, and 25-28 depend, directly or indirectly, on claims 1, 23, and 26, and are not obvious for at least the same reasons. Accordingly, withdrawal of the rejection to claims 1-4, 13-19, and 23-28 is respectfully requested.

Claims 1-4, 13-19, 21, and 23-28 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent in view of Ekins *et al.*, Analytica Chimica Acta (Analytica reference). This rejection is respectfully traversed.

As discussed above, the '202 patent does not teach an assay that requires a substantial depletion of the analyte from a sample and concentration of the analyte on the microscopic sorbent zones. The Analytica reference cannot remedy the defect of the '202 patent. As discussed in our previous response to the first Office Action, similarly to the Immunoassay reference, the Analytica reference discloses an ambient analyte immunoassay. It is required by the method that the proportion of bound analyte be so small that the "disturbance to the ambient analyte concentration can be ignored" (page 80, first paragraph). This "ignored" amount of the analyte depleted from the sample is further defined as "invariably less than 1% regardless of the analyte concentration" by the reference (page 80, first paragraph, last sentence). Therefore, like the Immunoassay reference, the Analytica reference does not teach or suggest the analyte being substantially depleted from the sample and concentrated on the sorbent zones. Instead, the Analytica reference teaches away from the present invention by requiring that only an insignificant amount of the analyte (less than 1%) is depleted from the sample. In light of the foregoing, applicants respectfully submit that the '202 patent and the Analytica reference, either alone or in combination, cannot make claims 1, 23, and 26 obvious. Claims 2-4, 13-19, 24, and 25 depend, directly or indirectly, on claims 1 and 23 and are not obvious for at least the same reasons. Accordingly, withdrawal of the rejection to claims 1-4, 13-19, 21, and 23-28 is respectfully requested.

Claims 5-10 are rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference, in further view of Ullman *et al.* (U.S. Patent 5,512,659). Claim 11 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in further view of Waggoner *et al.* (U.S. Patent 5,368,486). Claim 12 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in view of the Waggoner *et al.*, in further view of Lee *et al.* (U.S. Patent 5,453,505). Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over the '202 patent, and either the Immunoassay reference or the Analytica reference in view of Northrup *et al.* (U.S. Patent 5,639,423). Applicants respectfully traverse these rejections.

As discussed above, the '202 patent, the Immunoassay reference, and the Analytica reference, either alone or in combination, cannot make claim 1 obvious, because they teach away from the binding assay of the present application, which requires the analyte to be substantially depleted from the sample and concentrated on microscopic sorbent zones. Claims 5-10, 11, 12, and 20 depend, directly or indirectly, from claim 1 and cannot be made obvious by the '202 patent, the Immunoassay reference, and the Analytica reference for at least the same reasons.

Ullman *et al.*, Waggoner *et al.*, Lee *et al.*, and Northrup *et al.* cannot remedy the defect of the '202 patent, the Immunoassay reference, and the Analytica reference, and are not relied upon by the Examiner for such. Ullman *et al.*, Waggoner *et al.*, Lee *et al.*, and Northrup *et al.* have no teaching whatsoever of a binding assay utilizing a plurality of sorbent zones containing an analyte-binding partner, let alone a binding assay, which requires an excess of the analyte-binding partner relative to the analyte, so that any analyte present is substantially depleted from the sample and concentrated in the sorbent zones. Therefore, none of the cited references, either alone or in combination, can motivate one skilled in the art to arrive at claims 5-10, 11, 12, and 20. Withdrawal of the rejection is, therefore, respectfully requested.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles, California, telephone number 213-337-6700 to discuss the steps necessary for placing the application in condition for allowance.

Respectfully submitted,

HOGAN & HARTSON, LLP

Date: October 12, 2001

By:_____

Wei-Ning Yang
Registration No. 38,690
Attorney for Applicant(s)

500 South Grand Avenue, Suite 1900
Los Angeles, California 90071
Telephone: 213-337-6700
Facsimile: 213-337-6701